REMARKS

This paper is submitted in response to the Office Action mailed October 26, 2005 for the above-identified patent application. Claims 1-47 are pending in the application. Claims 20, 23-28 and 47 have been withdrawn from consideration. Claims 1-19, 21, 22 and 29-46 have been rejected.

Claims 1-10, 16, 19, 21, 22, 37 and 39 have been rejected under 35 U.S.C. 112 ¶1, as failing to comply with the enablement requirement. The Examiner alleges that the specification does not teach markers that are differentially present in TSE-infected subjects, other than cystatin C and isoforms of hemoglobin. In addition, the Examiner alleges that specification does not teach that markers are differentially present in body fluids other than cerebrospinal fluid (CSF) and plasma.

Applicants respectfully disagree. The Specification discloses markers of various molecular weights. For example, the specification discloses that a comparative study was undertaken between plasma from BSE-diagnosed cattle and normal plasma. The data demonstrates that the peaks of about 1010, 1100, 1125, 1365, 3645, 4030, 3890, 5820, 7520, 7630, 7980, 9950, 10250, 11600, 11800, 15000, 15200, 15400, 15600, 15900, 30000, 31000 and 31800 Da can be used to diagnose BSE in plasma samples. (*See* Specification, paragraphs 124-127). Furthermore, the specification discloses methods of determining these markers using surface-enhanced laser desorption/ionization (SELDI) (*See* Specification, Examples 6 and 7). Accordingly, Applicants respectfully submit that the disclosure of the present invention sufficiently identifies the claimed markers by molecular weight and/or protein name and discloses methods of determining the markers.

In addition, Applicants respectfully submit that the specification combined with knowledge of those skilled in the art, fully supports utilizing body fluids in addition to CSF and plasma that are taken from the subject. For example, proteins found in blood fractions, *e.g.*, plasma would also be expected in whole blood and serum. Furthermore, it is commonly known in the art that many proteins found in the blood will also be found in urine. For example, FABP is detectable in both blood and urine of patients after myocardial infarction. *See* Gorski, J. *et al.*, CLIN. CHEM, 43:193-195 (1997), copy attached.

For at least these reasons, reconsideration and withdrawal of the rejection of claims 1-10, 16, 19, 21, 22, 37 and 39 under 35 U.S.C. §112 ¶1, as failing to comply with the enablement requirement, is respectfully requested.

Claims 1-19, 21, 22 and 29-46 have been rejected under 35 U.S.C. 112 ¶2, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleges that the claims require that a diagnosis of TSE has been determined prior to the determination of the presence/absence of a polypeptide and comparison to a test amount.

In any diagnostic test a reference amount of a marker, *e.g.*, a polypeptide, is established for a 'normal' population. The amount of the marker for a 'diseased' population is also determined. Once these amounts, or ranges, are established it is possible to diagnosis a subject by simply measuring the amount of a marker and determining whether that amount is consistent with a 'normal' or 'diseased' population. Applicants respectfully submit that in view of the disclosure in the specification of

markers to identify TSE one skilled in the art would be able to apply the basic principle of diagnosing a patient, as recited in the claims. The claims do not require that a diagnosis of TSE has been determined prior to the determination of the presence/absence of a polypeptide. Instead, as presently claimed, the invention recites determining a test amount of a polypeptide in a sample *wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects*. The test amount of polypeptide in the sample is compared to a reference amount of polypeptide, which represents no TSE infection, and it is determined whether the test amount is consistent with a diagnosis of TSE.

Therefore, reconsideration and withdrawal of the rejection of claims 1-19, 21, 22 and 29-46 under 35 U.S.C. 112 ¶2, is respectfully requested.

Claims 1-19, 21, 22 and 29-46 have been rejected under 35 U.S.C. 112 ¶2, alleging that since only molecular weight ranges are given, it is unclear whether all of the components differentially contained in the tested samples are polypeptides. In particular, the Examiner states that if only the molecular weight is determined from mass spectrometry it cannot be ascertained if the substance is a polypeptide.

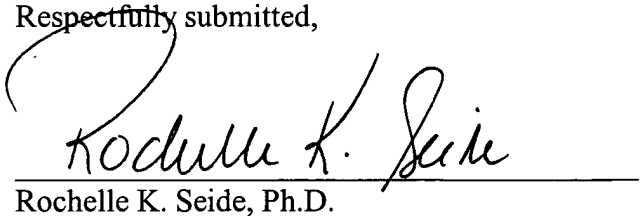
The specification discloses how to establish if the molecular weight determined from mass spectrometry is a polypeptide. (*See, e.g.*, Specification, Example 7). For example, an aliquot of the same sample used for SELDI is subjected to one-dimensional gel electrophoresis, a band of appropriate molecular weight is excised, digested with a protease (*i.e.*, a protein specific cleavage agent) and the polypeptides identified by mass spectrometry. Thus, the specification clearly teaches how to confirm that a biomarker

determined from mass spectrometry is a polypeptide and also identify which polypeptide. Furthermore, Ciphergen Biosystems Inc. markets a SELDI system that uses 'Protein Chips' to capture biomarkers and suggests a 'proteolysis' methodology to identify the biomarkers discovered on the 'Protein Chips.' (*See, e.g.*, Ciphergen Protein Chip System, at www.ciphergen.com, copy attached) Thus, the peaks in a SELDI profile are clearly due to the presence of polypeptides (*i.e.*, proteins). Accordingly, one skilled in the art would be able to determine if a molecular weight, determined from mass spectrometry, is a polypeptide.

For at least these reasons, reconsideration and withdrawal of the rejection of claims 1-19, 21, 22 and 29-46 under 35 U.S.C. 112 ¶2, is respectfully requested.

Applicants believe that no additional fees are required in connection with this response. However, if additional fees are required, the Commissioner is hereby authorized to charge any additional payment, or credit any overpayment, to Deposit Account No. 01-2300, **referencing Docket Number 108140.00030.**

Respectfully submitted,



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FEE CALCULATION

Any additional fee required has been calculated as follows:

X If checked, "Small Entity" status is claimed.

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT	(Column 2) HIGHEST NO. PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADD'L FEE	OR	LARGE ENTITY RATE	LARGE ENTITY ADD'L FEE
TOTAL CLAIMS	47 MINUS	**47**	**= 0**	x $25	**$0.00**		x $50	**$**
INDEP CLAIMS	17 MINUS	**17**	**= 0**	x $100	**$0.00**		x $200	**$**
☐ FIRST PRESENTATION OF MULTIPLE DEP. CLAIM				+ $180	**$0.00**	OR	+ $360	**$**
					$0.00			**$**

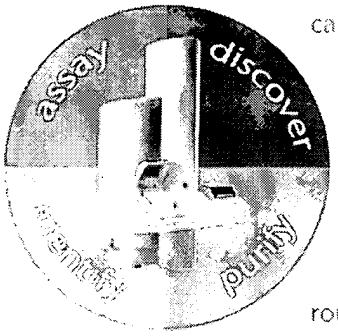
The U.S. Patent and Trademark Office is hereby authorized to charge and deficiency or credit any overpayment of fees associated with this communication to Deposit Account No. **01-2300** referencing docket number **108140.00030**.

ProteinChip® System, Series 4000

Accelerating biomarker discovery to assays

With over 1000 users world-wide and a large, rapidly growing citation list, Ciphergen's Surface Enhanced Laser Desorption/Ionization (SELDI) technology is the accepted leader in biomarker research. The ProteinChip System, Series 4000 is Ciphergen's newest generation of SELDI instrumentation designed, tested and manufactured incorporating 10 years of first-class biomarker experience.

Designed for today's biomarker research



Ciphergen's new ProteinChip System, Series 4000 incorporates the critical design features demanded by today's clinical researchers and biologists in biomarker research. The Series 4000 delivers improved performance from biomarker discovery to assay offering the fastest route to converting biomarker discoveries to biomarkers assays all on the same platform. This product note describes the revolutionary new features of this benchtop system, designed to fit into the research plans of any life science laboratory.

Outstanding quantitative biomarker capability

The Series 4000 delivers the best quantitative performance available for biomarker analysis. The system's sensitivity, dynamic range and reproducibility enable discovery and biomarker assays directly on the same platform.

ProteinChip System, Series 4000 Features

Superior quantitation	◦ *Raster laser design for maximum spot coverage* ◦ *Auto laser energy setting* ◦ *Improved ProteinChip Arrays and protocols* ◦ *Highest dynamic range*
Enhanced sensitivity	◦ *New high sensitivity detector* ◦ *New Ion Source increases ion efficiency* ◦ *New patented detector blanking reduces noise* ◦ *Innovative flight tube design*
Increased resolving power	◦ *Improved fractionation tools increase resolution up to 3000 proteins*
High throughput	◦ *Unattended runs of up to 168 ProteinChip Arrays*
Improved biomarker discovery	◦ *Pattern Track™ rapid biomarker discovery to assay* ◦ *Deep Proteome™-low abundance protein discovery* ◦ *Biomarker Pathways™-protein interaction pathway discovery & quantitative assays*

CIPHERGEN®

The ProteinChip® Company

Sensitivity

The Series 4000 instrument is the most sensitive laser desorption/ionization time-of-flight mass spectrometer available for protein and peptide analysis. It has high-attomole sensitivity for most peptides and many proteins. The Series 4000 is specially configured for sensitivity in the high mass range to allow detection of proteins above 100 kDa. For biomarker discovery and assays, this means you can confidently scan for proteins and peptides in a range from a few hundred Daltons to well over 200 kDa.

Sensitivity – superior detection of proteins above 100 kDa

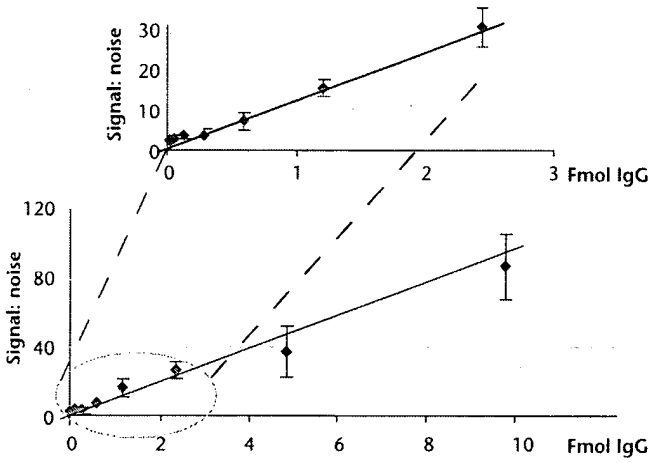


Figure 1: Detection of IgG (150 kDa) using the Series 4000: A quantitation range from 10 fmol to 180 attmol.

Typical reproducibility using the ProteinChip System, Series 4000

	TYPICAL CV%
Internal standard – biomarker assays	< 15%
External standard – biomarker assays	< 20%
Internal standard – interaction assays	< 10%
External standard – interaction assays	< 15%

Sensitivity Features

The ProteinChip System, Series 4000 achieves high sensitivity through the following features:

- *A patented conical ion source shape (CISS), efficiently directs desorbed peptides and proteins to the detector to detect the lowest signals possible.*
- *Synchronized Optical Laser Extraction (SOLE) — a raster scanning laser design ensures complete coverage of the ProteinChip Array spot for total desorption of the sample.*
- *A superior new detector system includes a patented detector blanking mechanism to reduce noise and eliminate detector saturation from off-scale signals such as the signal from the Energy Absorbing Molecule (matrix signal).*
- *The revolutionary flight tube design improves sensitivity by minimizing loss of ions as they travel to the detector, while the focusing ion optics maintains excellent resolution for peptide maps for protein identification*

Quantitation

The Series 4000 has been engineered to give reproducible results – so you can rely on your data every time! New pre-set calibrations for detector gain, laser energy metering and mass accuracy coupled with automated protocol features ensure high performance results for large scale biomarker studies. Using the fluid handling robotics system for sample preparation and Ciphergen's ProteinChip Arrays and quality reagents with the Series 4000 offers accuracy and reproducibility every single time!

Dynamic range

Most biological samples present the challenge that the proteins being studied exist in a very wide concentration range. It is reported that for serum analysis, the concentration range of proteins spans at least 10 orders of magnitude; considering albumin (more than 50% of the protein content of serum) down to the lowest abundance proteins observed to date. The concentration for any given biomarker may vary by several orders of magnitude amongst a sample population as well. The Series 4000 has a significantly extended dynamic range analysis capability to meet this challenge.

The system's state-of-the-art electronics maximize the range of protein detection so that the only limitations of dynamic range result from chemical phenomenon. Coupled with Ciphergen's high performance ProteinChip Arrays which concentrate proteins of interest several orders of magnitude, the system is capable of quantifying most biomarkers in the attomole range (pg-ng/mL depending on molecular weight). The availability of high resolution pre-fractionation kits and protocols combined with the ProteinChip System, Series 4000 provides the complete solution for analysis of low abundance proteins in serum.

Quantitation Features

The ProteinChip System, Series 4000 achieves reproducible quantitation through the following features:

- *SOLE – raster scanning laser design covers the entire ProteinChip Array spot to produce reproducible results*
- *Automatic settings for detector gain and laser intensity and self-calibrating electronics ensure consistent performance*
- *Automated protocols using robotic fluid handling systems for sample preparation*
- *Reproducible ProteinChip Arrays produced in Ciphergen's state-of-the-art automated manufacturing facilities*

Dynamic range of the ProteinChip System, Series 4000

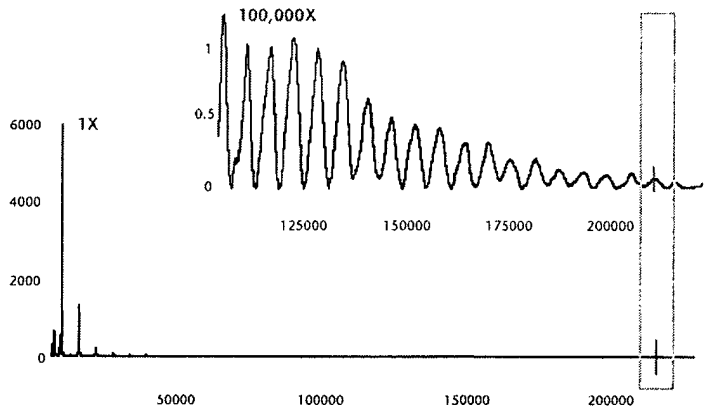


Figure 2: Detection of abundant and low-abundance insulin species. At 1x magnification, the insulin monomer at 5.7 kDa and the insulin multimer (36-mer) at >200 kDa are both on scale. Detection of the multimeric insulin species is shown at a magnification of 100,000x. The intensity ratio of the insulin monomer to the insulin multimer is 1:85,000.

Protein & peptide resolution

Ciphergen's bead-based reagents, and methods for serum pre-fractionation, together with ProteinChip Arrays, dramatically increase resolution of the number of proteins and peptides that can be detected. Coupled with the improved dynamic range offered by the Series 4000, these methods and reagents add up to the most comprehensive resolution of complex proteome samples.

Spotting neat samples onto arrays typically results in in 50-100 proteins. Applying pre-fractionation tools increases this to 3,000 proteins.

Resolution Features

The ProteinChip System, Series 4000 achieves increased resolution through the following features:

- *Multiple ProteinChip Array chemistries selectively retain subsets of proteins from complex samples*
- *Pre-fractionation technologies to further enrich lower abundance proteins enabling access to the Deep Proteome*
- *Series 4000 innovative electronics maximize the dynamic range of the instrument to allow detection of a larger number of proteins*

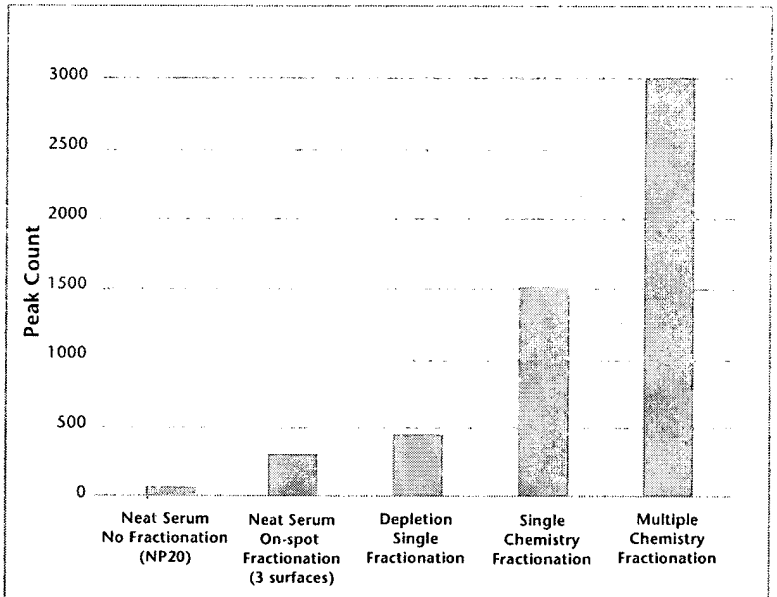


Figure 3: Improving resolution of a greater number of proteins using pre-fractionation technology.

ProteinChip System, Series 4000 Software

Included with the ProteinChip System, Series 4000, Ciphergen's powerful software solutions provide the fastest, most effective route for dealing with the large amounts of data generated during biomarker studies. The custom-designed tools include up front sample and data tracking integrated with sophisticated biostatistical analysis packages.

CIPHERGENEXPRESS™ DATA MANAGER & BIOMARKERS ANALYSIS MODULE

The Data Manager module includes a robust client-server relational database system for management and tracking of SELDI data. A flexible Biomarker Analysis module provides powerful data mining and analysis capabilities for rapid, automated analysis of multiple experiments over multiple conditions in large project groups for delineation of potential biomarkers.

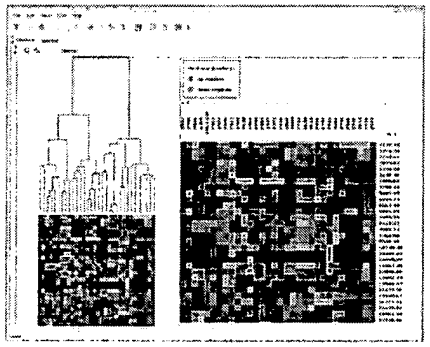
BIOMARKER PATTERNS™ SOFTWARE

Biomarker Patterns Software (BPS) quickly discovers hidden patterns in SELDI data sets to uncover complex relationships. Using the biostatistical procedure, CART (Classification and Regression Trees) multiple biomarkers are correlated with specific phenotypes to improve sensitivity and specificity over single markers. The output is an easy to interpret decision tree, using a small panel of markers with defined splitting rules. The software translates the discovery of multiple markers into highly predictive biomarker assays.

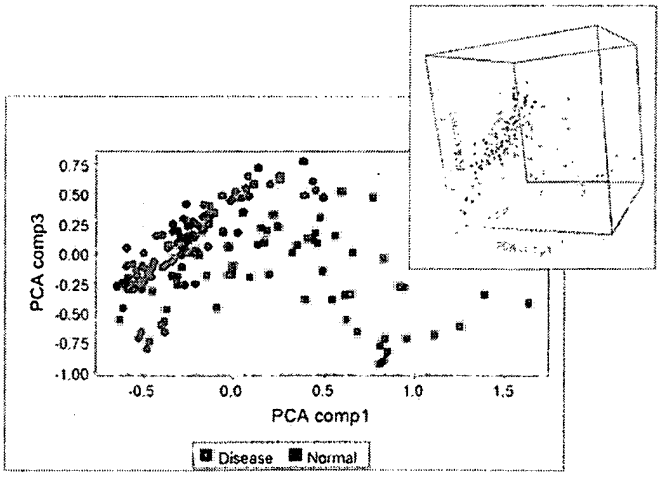
Data Management Features

The ProteinChip System, Series 4000 manages and analyzes data through the following features:

- *Bar-coded arrays, and Virtual Notebook feature allow for efficient sample and protocol tracking*
- *Robust client-server database centralizes data and ensures data integrity*
- *Intuitive user-interface provides ease of use for both instrument control & data analysis*
- *Ability to control the instrument from a remote location*
- *BPS determines multiple biomarker correlation with sample sets in clinical research studies to improve sensitivity and specificity over single marker methods*



Hierarchical clustering: The heat map provides a relative-expression view of spectra. Red indicates increased expression and green indicated reduced expression.

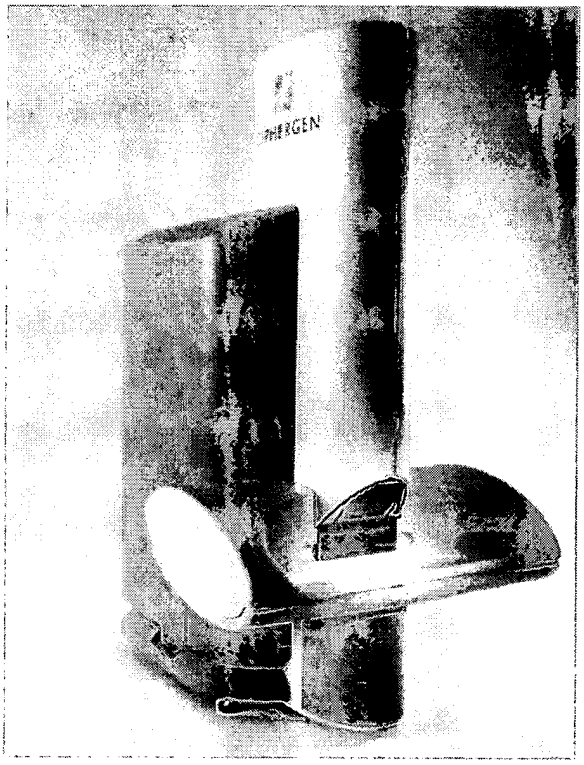


Principal component analysis: This multivariate analysis tool provides two- and three- dimensional graphic visualization of complex relationships between variables.

ProteinChip System, Series 4000 Personal Edition

ADVANCED PERFORMANCE
FOR BIOMARKER RESEARCH
AT YOUR LAB BENCH!

The ProteinChip System, Series 4000 – Personal Edition is designed with the biology laboratory in mind. The Personal Edition offers affordable, benchtop, biomarkers research for academic and industrial laboratories. The Personal Edition includes the CISS Ion source and SOLE raster scanning laser design for maximum sensitivity and detection, and the dynamic range capabilities of the Personal Edition are identical to the Enterprise Edition. The Personal Edition can be fully upgraded to an Enterprise Edition at any time.



The ProteinChip System, Series 4000 Personal Edition.

System configuration

The ProteinChip System, Series 4000 – Personal Edition is designed for complete biomarker discovery, characterization and assay development in low to medium throughput labs. This system includes all of the superior engineered features for sensitivity, dynamic range and quantitation. The complete package includes:

- *The Series 4000 Personal Instrument*
- *CiphergenExpress Data Manager – Personal Edition*
- *Starter Kit package of arrays and reagents*
- *Biomarker discovery and assay tools*
  - *Expression Difference Mapping™ kits – for serum fractionation and biomarker profiling*
  - *IDM Affinity Beads – for interaction discovery mapping studies*
  - *BioSepra chromatographic sorbents – for biomarker purification*

ProteinChip System, Series 4000 Enterprise Edition

HIGH THROUGHPUT: COLLECT THOUSANDS OF SAMPLES AT ONCE!

The Series 4000 – Enterprise Edition is designed for high throughput biomarker analysis research. Thousands of samples can be analyzed, unattended, using ProteinChip Arrays each with a barcode for automated data tracking.

- *Automated ProteinChip Array loading – 12 ProteinChip Arrays per cassette,* ***up to 14 cassettes (168 arrays) at one time!***
- *Built-in barcode scanner for ProteinChip Arrays*
- *CiphergenExpress Software for data tracking, data management and data analysis*

REMOTE ACCESS FOR LABORATORY INFORMATION MANAGEMENT

The Series 4000 – Enterprise Edition has built-in networking capabilities for linking to existing Laboratory Information Management Systems (LIMS), and the instrument can be controlled from a remote site by a designated user.

- *Run the instrument from your workstation!*
- *Networking capabilities*
- *For extended large-scale biomarker projects*

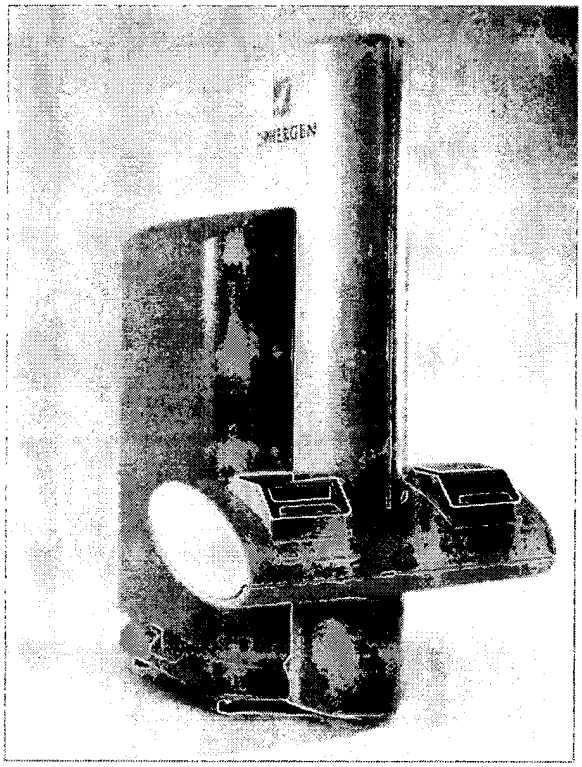
System configuration

The ProteinChip System, Series 4000 – Enterprise Edition is a fully automated system for biomarker discovery and assay. The complete package includes:

- *Series 4000 Enterprise Instrument*
- *CiphergenExpress Data Manager*
- *14 cassette AutoLoader capacity to automatically feed up to 168 ProteinChip Arrays*
- *Starter Kit package of arrays and reagents*

Additional applications and automated array preparation packages available include:

- *Automated Laboratory Workstation with ProteinChip Integration Package*
- *CiphergenExpress Biomarker Analysis Package – analysis software*
- *Biomarker Patterns Software – classification and regression tree (CART) analysis software*
- *Biomarker discovery and assay tools*
  - *Expression Difference Mapping kits – for serum fractionation and biomarker profiling*
  - *IDM Affinity Beads – for interaction discovery mapping studies*
  - *BioSepra chromatographic sorbents – for biomarker purification*



The ProteinChip System, Series 4000 Enterprise Edition.

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Product ordering information

ProteinChip® System, Series 4000 Personal Edition	Z500-0013/23
ProteinChip System, Series 4000 Enterprise Edition	Z500-0012/22
ProteinChip System, Series 4000 Enterprise Biomarker Edition	Z500-0011/21
ProteinChip System, Series 4000 Enterprise AutoBiomarker Edition	Z500-0010/20

Related information

CIPHERGEN LITERATURE

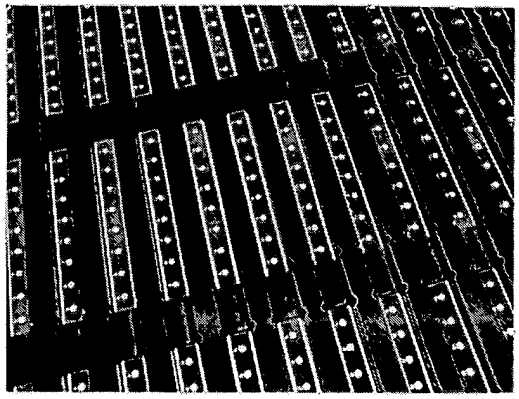
ProteinChip System, Series 4000
Technical Note

CIPHERGEN WEBSITE

A plethora of related information is available on Ciphergen's website.

For related citations, posters, papers and product literature go to:
www.ciphergen.com/pub/searchPub.asp

For technical documents reserved for users only or to participate in the online discussion group on SELDI technology and its application, go to:
www.ciphergen.com/users



About Ciphergen Biosystems

Ciphergen develops, manufactures and markets ProteinChip® Systems that enable protein discovery, characterization and assay development so researchers can gain a better understanding of biological functions at the protein level.

The ProteinChip Systems are novel, enabling tools that provide a direct approach to understanding the role of proteins in the biology of disease, monitoring of disease progression and the therapeutic effects of drugs.

Pioneering researchers are now taking full advantage of Ciphergen's powerful SELDI-based ProteinChip platform to advance clinical proteomics for predictive medicine.

LPN-0104 Rev 002 5M 0804

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* Author for correspondence.

Increased Fatty Acid-Binding Protein Concentration in Plasma of Patients with Chronic Renal Failure

To the Editor:

The soluble cytoplasm of most cells contains low-molecular-mass (14–15 kDa) proteins able to bind long-chain unesterified fatty acids. Of these so-called fatty acid-binding proteins (FABP), nine different types have been identified *[1, 2]*. Heart and skeletal muscles contain the same type of FABP [referred to as heart-type (H)-FABP] *[1, 2]*, but its concentration in the heart is severalfold higher than that in the skeletal muscles *[3]*. The concentration of FABP in the plasma of healthy persons is relatively low (2–6 $\mu g \cdot L^{-1}$) *[4]*. FABP is released from the heart early after the onset of infarction, whereafter its plasma concentration increases manyfold *[3–6]*. Increased excretion of FABP in urine also occurs after infarction *[5, 7]*. Several recent studies indicate the usefulness of the plasma FABP concentration as an early biochemical marker for myocardial infarction diagnosis *[3, 5, 7]*. However, to interpret properly the values of plasma

FABP concentration, one has to take into account not only its source and rate of release into plasma but also its elimination from plasma. It is obvious that any change in the clearance rate of FABP would affect its plasma concentration, and thus may lead to erroneous interpretation. Kleine et al. *[8]* reported a patient with acute myocardial infarction and severe renal insufficiency in whom the plasma FABP concentration remained increased for the whole course of blood sampling (25 h after the infarction), whereas in patients with normal kidney function it normalized in ~10 h after the infarction. Unfortunately, preinfarction data on plasma FABP in this patient were not available. Low-molecular-mass proteins such as FABP and myoglobin are cleared mostly by the kidney *[9, 10]*. As it remains an open question whether, and, if so, to what extent an insufficiency of the kidneys affects the plasma FABP concentration in patients with heart and skeletal muscles intact, we studied plasma FABP and myoglobin in patients with chronic renal failure.

Blood samples were obtained from 15 blood donors (males) and 27 chronically hemodialyzed patients with renal failure (18 males, 9 females, ages 17–66 years; period of dialysis 2–70 months). Their primary renal diseases were: chronic glomerulonephritis (n = 14), interstitial nephritis (n = 2), acute renal failure (n = 3), adult dominant polycystic kidney disease (n = 3), hypertensive nephropathy (n = 3), diabetes mellitus (n = 1), and amyloidosis (n = 1). The patients were clinically stable and free of any severe intercurrent illnesses. They had no clinical evidence of severe secondary hyperparathyroidism. Hemodialysis was performed three times a week with the double needle technique, with cuprophane capillary dialyzers, and with bicarbonate as buffer in the dialysate. The membrane allows the passage of low-molecular-mass solutes up to ~2 kDa. Vascular access was in all cases a Cimino-Brescia arteriovenous fistula. Blood samples were obtained immediately before and after dialysis.

Plasma FABP concentration was measured by a sensitive noncompetitive sandwich ELISA *[4]*. Plasma concentration of myoglobin was measured with a turbidimetric immunoassay (Unimate 3 MYO, Roche Diagnostic Systems, Basel, Switzerland) on a Cobas Mira Plus analyzer (Roche). The concentrations of urea and creatinine in plasma were measured by the urease method and Jaffe reaction, respectively.

The significance of the differences between the means was evaluated statistically by unpaired and paired Student *t*-tests, where appropriate. Correlations between plasma FABP and (or) myoglobin concentrations and the period of dialysis, and plasma urea and creatinine concentrations were determined by Pearson product-moment correlation, and the level of significance was taken at $P < 0.05$.

Plasma creatinine and urea concentrations were high before dialysis and dropped markedly after dialysis (Table 1). The mean plasma concentration of FABP in the uremic patients before and after dialysis was 21 and 25 times higher, respectively, than that in the blood donors. The mean plasma myoglobin concentration in the uremic patients before and after dialysis was 3.7 and 4.0 times higher, respectively, than that in the blood donors. The insignificant increase in plasma concentrations of FABP and myoglobin after dialysis may reflect removal of blood water during dialysis. In the patients, before dialysis the mean myoglobin/FABP ratio was five times lower than in the donors, and after dialysis six times lower (Table 1). Neither plasma FABP nor plasma myoglobin concentrations showed a correlation with the period of dialysis or urea or creatinine concentration in plasma.

The present data are the first to show that plasma FABP concentration is markedly increased in patients with chronic renal failure and normal heart function, similar to that found for myoglobin *[11]*. It is clear that a certain amount of each protein must be constantly removed either by the kidney or by other tissues, thus preventing progressive increase in the concentration with time of renal failure. Interestingly, the plasma FABP concentration is much higher (20–25 fold) than that of myoglobin (fourfold) despite the fact that these proteins have similar molecular masses (15 and 18 kDa, respectively) and show a similar plasma release curve in patients with acute myocardial infarction and normal renal function *[3]*. These findings suggest that the kidneys play a more dominant role in the clearance of plasma FABP than of myoglobin.

The ratio of the concentrations of myoglobin over that of FABP is lower in the heart (ratio ~5) than in skeletal muscles (20–70, depending on muscle type) *[3]*. The use of the ratio of the plasma concentrations of myoglobin over that of FABP to discriminate between heart and skeletal muscle tissue injury has been sug-

Table 1. Plasma FABP and myoglobin concentrations in controls and patients with chronic renal failure.

Subjects		Creatinine, mg %	Urea, mg %	Myoglobin $\mu g \cdot L^{-1}$ (range)	FABP, $\mu g \cdot L^{-1}$ (range)	Myoglobin/FABP (ratio)
Controls (n = 15)		0.77 ± 0.14	25.1 ± 7.7	46.8 ± 20.7 (22.3–96.8)	3.0 ± 1.4 (1.4–5.0)	16.2 ± 4.3 (10.9–25.12)
Renal failure patients (n = 27)	B	11.4 ± 3.3	118.0 ± 30.2	170.6 ± 61.8 (63.8–290.1)	62.8 ± 25.2 (32.1–118.2)	3.2 ± 1.8 (0.7–9.0)
	A	4.6 ± 1.3	53.9 ± 18.6	181.3 ± 67.6 (70.2–297.6)	75.5 ± 28.9 (13.6–120.9)	2.7 ± 1.2 (1.2–5.7)

B, before dialysis; A, after dialysis.

gested [3]. Because of the relatively longer [illegible] of plasma FABP compared with myoglobin, the ratio calculated for uremic patients ([illegible]) is similar to that found in patients after heart infarction. Thus, with respect to the discrimination of myocardial from skeletal muscle injury, the decrease of the ratio in chronic renal failure indicates the limitation of the use of this ratio for this purpose.

Serial monitoring of the plasma FABP concentration can also be used to estimate infarct size [6]. However, our results indicate that if the myocardial infarction occurred in a patient with chronic renal failure, the plasma FABP concentration would be relatively higher than in a patient with intact kidneys, thus leading to overestimation of infarct size. Since preinfarct values differ widely among patients, a judgment about infarct size cannot be made.

In conclusion, our data indicate that in patients with chronic renal failure the plasma concentrations of the biochemical markers FABP and myoglobin each are markedly increased. Thus, caution must be taken when using these marker proteins for early diagnosis of myocardial infarction in case of renal insufficiency, as the preinfarct plasma concentration is very likely to be already high.

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References

1. Glatz JFC, Borchers T, Spener F, Van der Vusse GJ. Fatty acids in cell signalling: modulation by lipid binding proteins. Prostaglandins Leukot Essent Fatty Acids 1995;52:121–7.
2. Van Nieuwenhoven FA, Van der Vusse GJ, Glatz JFC. Membrane associated and cytoplasmic fatty acid-binding proteins. Lipids 1996;31:S223–7.
3. Van Nieuwenhoven FA, Kleine AH, Wodzig KWH, Hermens WT, Kragten HA, Maessen JG, et al. Discrimination between myocardial and skeletal muscle injury by assessment of the plasma ratio of myoglobin over fatty acid-binding protein. Circulation 1995;92:[illegible]
4. Wodzig KWH, Pelsers MMAL, Van der Vusse GJ, Roos W, Glatz JFC. One-step enzyme-linked immunosorbent assay (ELISA) for plasma fatty [illegible]
5. [illegible] T, [illegible], [illegible] Fatty acid-binding protein as [illegible] of acute myocardial infarction. Clin Biochem 1991;24:195–201.
6. Glatz JFC, Kleine AH, Van Nieuwenhoven FA, Hermens WT, Van Dieijen-Visser MP, Van der Vusse GJ. Fatty acid-binding protein as a plasma marker for the estimation of myocardial infarct size in humans. Br Heart J 1994;71:135–40.
7. Tsuji R, [illegible] T, [illegible], Yoshimoto K, [illegible] K, et al. Human heart-type cytoplasmic fatty acid-binding protein in serum and urine during hyperacute myocardial infarction. Int J Cardiol 1993;41:209–17.
8. Kleine AH, Glatz JFC, Van Nieuwenhoven FA, [illegible]. Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. Mol Cell Biochem 1992;116:155–62.
9. [illegible] C, [illegible]. [illegible] proteinuria. Ann Rev Med 1979;30:[illegible]
10. [illegible], [illegible] DC. Renal uptake and disposal of proteins and peptides. In: [illegible], eds. Biological barriers to protein delivery. New York: Plenum Press, 1993:[illegible]–336.
11. [illegible] HC. Myoglobin, an early biochemical marker for the diagnosis of acute myocardial infarction. J Clin Immunoassay 1994;17:[illegible]–29.

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